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Insights into plant water uptake from xylem-water isotope measurements in two tropical catchments with contrasting moisture conditions

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Abstract:

Water transpired by trees has long been assumed to be sourced from the same subsurface water stocks that contribute to groundwater recharge and streamflow. However, recent investigations using dual water stable isotopes have shown an apparent ecohydrological separation between tree-transpired water and stream water. Here we present evidence for such ecohydrological separation in two tropical environments in Puerto Rico where precipitation seasonality is relatively low and where precipitation is positively correlated with primary productivity. We determined the stable isotope signature of xylem water of 30 mahogany (*Swietenia* spp.) trees sampled during two periods with contrasting moisture status. Our results suggest that the separation between transpiration water and groundwater recharge/streamflow water might be related less to the temporal phasing of hydrologic inputs and primary productivity, and more to the fundamental processes that drive evaporative isotopic enrichment of residual soil water within the soil matrix. The lack of an evaporative signature of both groundwater and streams in the study area suggests that these water balance components have a water source that is transported quickly to deeper subsurface storage compared to waters that trees use. A Bayesian mixing model used to partition source water proportions of xylem water showed that groundwater contribution was greater for valley-bottom, riparian trees than for ridge-top trees. Groundwater contribution was also greater at the xeric site than at the mesic–hydric site. These model results (1) underline the utility of a simple linear mixing model, implemented in a Bayesian inference framework, in quantifying source water contributions at sites with contrasting physiographic characteristics, and (2) highlight the informed judgement that should be made in interpreting mixing model results, of import particularly in surveying groundwater use patterns by vegetation from regional to global scales.
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INTRODUCTION

The partitioning of infiltrating water between plant transpiration, soil water evaporation, groundwater recharge, and streamflow generation under different physiographic characteristics is poorly understood (Vivoni *et al*., 2008; Gouet-Kaplan *et al*., 2012). While advances in terrestrial ecohydrology have improved our appreciation of the role of vegetation in modifying seasonal macroclimates (Bonan *et al*., 2002; Lee *et al*., 2005; Troch *et al*., 2009) and local microclimates (Simonin *et al*., 2013; Green *et al*., 2015), the prevailing assumption is that vegetation draws water from the same subsurface stocks that eventually reach the stream, i.e. green water flows and blue water flows (D'Odorico *et al*., 2010) originate from the same homogeneous source. If that were universally the case (knowing plant-water uptake is generally a non-fractionating process; Ehleringer and Dawson, 1992; Zimmermann *et al*., 1966), then subsurface water pools contributing to groundwater recharge and streamflow should have similar stable water isotope ratios to plant xylem water. However, work by Brooks *et al*. (2010) and Goldsmith *et al*. (2012) in Mediterranean and seasonally tropical settings, respectively, has suggested that there may be ecohydrological separation of the water sources for streams and trees, in that plants typically use matrix soil

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water not contributing to streamflow, while the water contributing to streamflow is not accessed by the plants (McDonnell, 2014). More recently, global-in-scale investigations (Evaristo *et al*., 2015; Good *et al*., 2015) have shown that a poorly mixed (i.e. ecohydrological separation) conceptualization of soil water pools is more likely the rule than the exception, and that water contributing to groundwater recharge is often (but not always) isolated from water used in plant transpiration (Jasechko *et al*., 2014; Jasechko and Taylor, 2015).

Evaristo *et al*. (2015) showed that ecohydrological separation was greatest in tropical and Mediterranean biomes. Most of the tropical studies in the meta-analysis of Evaristo *et al*. (2015), however, were in highly seasonal climates. Climates with less seasonality, where temporal contrasts in water availability and primary productivity (i.e. soil water uptake) are not as marked as in highly seasonal tropical settings, are particularly in need of study. The exchange between soil-matrix and preferential flow path waters may be more frequent at low-seasonality sites [i.e. they show greater wetness interconnectivity; Phillips, 2010]. Therefore, we hypothesize that the degree of ecohydrological separation would be less for ecosystems where rates of precipitation input and primary productivity are more in-phase.

Here we test for evidence of ecohydrological separation using similar species of mahogany trees (*Swietenia spp.*) at two low-seasonality but contrasting sites in northeastern and southwestern Puerto Rico, having significant differences in rainfall amount as well as atmospheric evaporative demand (i.e. potential evapotranspiration, PET). The site in the northeastern part of the island (Luquillo, hereafter LUQ) is a mesic–hydric ecosystem with ample rainfall throughout the year; the site in the southwest (Susua, hereafter SUS) represents a xeric ecosystem with about a fifth of the rainfall amount received in LUQ. Despite these contrasts in overall moisture regime, each site has little seasonal variation in terms of temperature and day length so that within-site hydrologic (e.g. precipitation, soil moisture) and primary productivity variability are in-phase. The mesic–hydric and xeric sites remain relatively wet and dry, respectively, on intra- and inter-annual timescales (see Figure 1). Thus, the sites provide an opportunity to test for the ecohydrological separation hypothesis under conditions where hydrology and primary productivity are in-phase, and where there is a significant contrast in rainfall amount and PET between sites. We anticipated the results would also provide information on the degree of wetness interconnectivity (exchange between matrix water and preferential flow water) at the sites.

We also explore the partitioning of water sources as an integrated signal in the xylem. Heretofore, much of the work in stable isotope tropical ecohydrology has centred on source water partitioning approaches that examine relationships between leaf phenology, differences in rooting patterns, and root activity (Meinzer *et al*., 1999) among other plant and environmental variables (Jackson *et al*., 1995; Stratton *et al*., 2000; Andrade *et al*., 2005; Gutierrez-Soto and Ewel, 2008; Rossatto *et al*., 2012; Bertrand *et al*., 2014). Stable isotope methods used in partitioning source contributions to xylem water fall under two main categories: process-based mixing (PBM) models and simple linear mixing (SLM) models (see Ogle *et al*., 2014). PBM models (e.g. *RAPID* by Ogle *et al*., 2004; Ogle *et al*., 2014) integrate stable isotope data and a biophysical model (e.g. root water uptake) into a Bayesian framework. PBM models are useful if the goals are to arrive at greater predictive ability of how changes in space and time affect root water uptake and an improved mechanistic understanding of ecosystem behaviour. Traditional SLM models are useful in estimating two or three water sources (e.g. Thorburn and Walker, 1993; Brunel *et al*., 1995). Relatively recent SLM models can deal with multiple sources via an iterative mass balance approach (e.g. *IsoSource* by Phillips and Gregg, 2003) or when used in a Bayesian inverse modelling framework (e.g. *MixSIR* by Moore and Semmens, 2008; *SIAR* by Parnell *et al*., 2010). There have been few plant source water partitioning studies using SLM models in a Bayesian framework, however, (e.g. Leng *et al*., 2013; Barbeta *et al*., 2015), and in this paper we examine the usefulness of this approach.

Specifically, we address the following questions:

1. Do analyses of stable isotopes in stream water, groundwater, bulk soil water, and plant xylem water for the contrasting wet- and dry-climate sites show evidence of ecohydrological separation?
2. What can we learn from a SLM model, implemented in a Bayesian inference framework, regarding the sources of water for the sampled mahogany trees?

We utilized the natural abundances of hydrogen (^{2}H or deuterium, D) and oxygen (^{18}O) stable isotopes in plant xylem water, and derived line-conditioned excess (lc-excess*) (Landwehr and Coplen, 2006) to test the ecohydrological separation at the two sites. The lc-excess* can help to differentiate water samples that have undergone evaporation under non-equilibrium conditions (Dansgaard, 1964) from those that have maintained the isotopic characteristics of regional precipitation. By using the lc-excess* to differentiate between evaporated (shallow soil water, standing water) and non-evaporated (precipitation, stream and groundwater) sources, we can test the ecohydrological separation. Finally, we compare estimates of the potential sources of xylem water by

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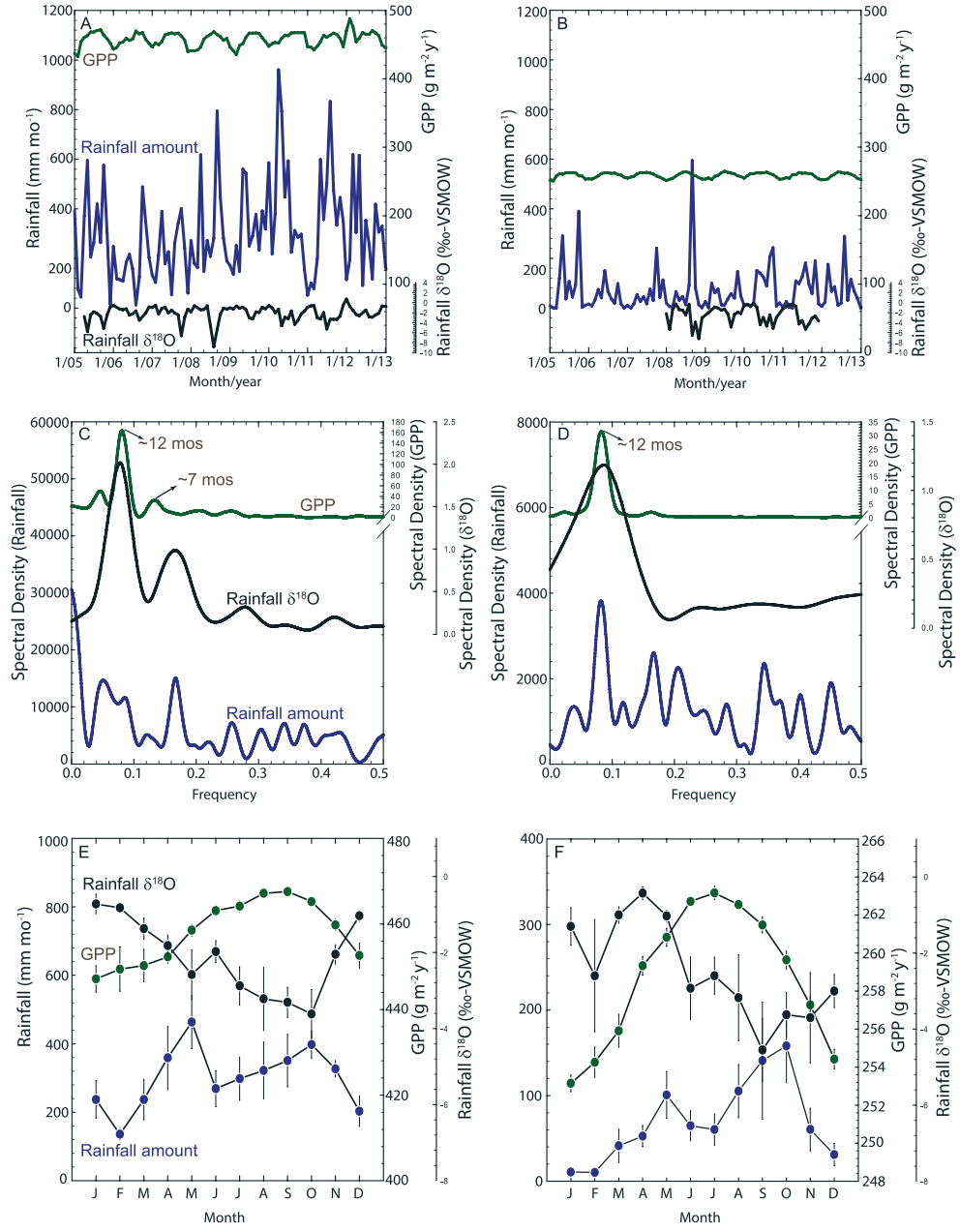

Figure 1. Demonstrating 'in phase' correspondence between hydrology (precipitation) and ecology (primary productivity). (A) and (B) Monthly GPP, rainfall amount, and rainfall isotope between January 2005 and June 2013 in LUQ and SUS, respectively. (C) and (D) Power Spectral Density analysis of GPP, rainfall amount, and rainfall isotope in LUQ and SUS, respectively. (E) and (F) Long-term, monthly means (error bars are standard error) of GPP, rainfall amount, and rainfall isotope over the same 2005–2013 period

exploring the utility of a SLM model, implemented within a Bayesian framework (*SIAR*, Parnell *et al*., 2010).

MATERIALS AND METHODS

Study area

The Luquillo Mountains in northeastern Puerto Rico rise steeply from the coast to over 1000 m in elevation over a distance of 15 to 20 km. They are characterized by steep slopes, rugged peaks, and highly dissected valleys (Pike *et al*., 2010). The rapid increase in elevation corresponds to major changes in climate, soil type, as well as structure and species composition of the vegetation (Scatena and Lugo, 1995). The site in the Luquillo Mountains (LUQ) chosen for this study was Rio Chiquito near Sabana (18°19′N, 65°43′W) at an elevation of approximately 160 to 207 m above sea level (Table I). The site is underlain by volcaniclastic rocks (tuffaceous sandstones and indurated siltstones) that have weathered

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Table I. Summary of site characteristics

Site name[a]	Elevation (masl)	MAP[b] (mm y^{-1})	Mean annual RH[b] (%)	Lifezone and geology[c]	Topography[d]	Land use	Mahogany species[e]
LUQ	160–207	3700	86.4	Wet; volcanic sedimentary	R, S, V	Protected forest	Big-leaf (*S. macrophylla*)
SUS	132–172	1200	65.5	Dry; serpentine	R, V	Protected forest	Small-leaf (*S. mahagoni*)

[a] LUQ = Luquillo; SUS = Susua.
[b] MAP = mean annual precipitation; RH = relative humidity; Sources: Luquillo Critical Zone Observatory website for LUQ, Weather Underground for SUS between 2004 and 2012.
[c] Puerto Rico falls within the subtropical belt of the Holdridge Life Zone System (Helmer *et al*., 2002).
[d] R, S, and V mean ridge, slope, and valley, respectively.
[e] Hybrid (*S. macrophylla* × *S. mahagoni*) also present in LUQ.

into a predominantly clayey substrate. Soils at this site are Typic Haplohumults of the Humatas Series with a solum thickness between 56 and 130 cm (USDA-NCSS, 2002) that is underlain by saprolite down to 20–60 m depth (Buss *et al*., 2013). The uppermost 20 cm of the soil is highly permeable but the soil below is rather poorly drained. As a result, most stormflow travels laterally through macropores in the topsoil (Schellekens *et al*., 2004). The LUQ site is part of the Tabonuco (*Dacryodes excelsa*) forest type (Wadsworth, 1951), a forest community found at elevations <600 m, with an average canopy height of 20–25 m. While no definitive survey on rooting depth exists for *Swietenia* spp. in Puerto Rico, Lugo *et al*. (2003) reported that mahogany trees at LUQ are less resistant to wind stress, possibly because of their relatively shallow rooting pattern. A soil survey by the USDA-NCSS (2002) reported medium-sized and fine roots (tree species not identified) down to a depth of almost 1 m at our site in LUQ. Most roots, however, were reported to be in the top 0.24–0.40 m (Lenart *et al*., 2010). The semi-deciduous hardwood species *Swietenia macrophylla* × *S. mahagoni* – a hybrid between small- and big-leaf mahoganies – was introduced in Luquillo more than 50 years ago (Lugo, 1992) and remains abundant in some parts of the Tabonuco forest including the present study site. The climate is maritime tropical (type A2m in the Köppen classification) with a mean annual rainfall (1988–2002) of ca. 3700 mm (Heartsill-Scalley *et al*., 2007) distributed over 267 rain days (Schellekens *et al*., 2000) while air temperatures vary seasonally between 22 and 25 °C. The site is exposed to the NE trade winds and receives relatively higher rainfall in the months of May, June, and October (>300 mm each) than at other times of the year while January through April typically have relatively low rainfall (<200 mm $month^{-1}$) (Heartsill-Scalley *et al*., 2007). Potential evapotranspiration (PET) according to the method of Hargreaves and Allen (2003) is ca. 1450 mm y^{-1} (Beck *et al*., 2013).

Situated in the southwestern part of the island and on the leeward side of the Cordillera Central, Susua (SUS) has a much drier climate than Luquillo. The study site is located on the southern extreme of the Susua Forest Reserve, along the banks and upper slopes of the Rio Loco (18°04′N, 66°54′W) at an elevation of 132–172 m (Table I). Mean annual rainfall is estimated to be 1200 mm (Medina *et al*., 1994) and air temperature varies seasonally between 25 and 29 °C. Like LUQ, January through April typically have lower rainfall than the rest of the year. Annual PET according to the Hargreaves method is estimated at ca. 1650 mm. The landscape is underlain by serpentinite that has weathered into the clayey, ferruginous, shallow Typic Hapludox of the Rosario Series – well-drained, moderate to rapidly permeable soils on side slopes and stable ridges with no aquic conditions for most of the year (USDA-NCSS, 2002). The plantation species *Swietenia mahagoni* (small-leaf mahogany) was introduced in SUS more than 50 years ago (Lugo, 1992) and remains abundant in the area. Like the hybrid mahogany at LUQ, small-leaf mahogany in SUS is facultatively deciduous – leaf shedding may be deferred or reduced to a rapid leaf replacement when sufficient soil moisture persists during the drier months (Burton, 2007). Unlike the hybrid mahogany at LUQ, small-leaf mahogany trees at SUS show more resistance to wind stress, possibly because of a deeper rooting pattern (Lugo *et al*., 2003).

Ecohydrological seasonality

A prime motivation for our study was testing the ecohydrological separation hypothesis in a setting where plant ecology (i.e. primary productivity) and site hydrology (i.e. moisture input) are in-phase, and where hydrological seasonality (in terms of precipitation inputs and streamflow outputs) is lower than at previously studied sites. To demonstrate that our Puerto Rico sites meet these conditions we performed a time-series analysis of rainfall (as a metric of hydrological conditions) and

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gross primary productivity GPP (as a metric of ecosystem performance) for the period January 2005 to June 2013.

We calculated GPP by first estimating the above-ground net primary productivity (ANPP) using the empirical relationship between ANPP and evapotranspiration of Webb *et al*. (1978). Because direct measures of below-ground net primary productivity (BNPP) are mostly lacking for tropical forests, we used the lower and upper bound estimates of Clark *et al*. (2001) [BNPP = 0.2–1.2(ANPP)] to calculate BNPP, and therefore obtain a first estimate of total NPP (i.e. ANPP + BNPP). We tested the validity of this approach by comparing our calculated total NPP to reported values in the literature for our study sites: Wang *et al*. (2003) for LUQ and Murphy *et al*. (1995) for SUS. We then calculated GPP (GPP = total NPP + respiration) by using reported values in the literature for the relationship between respiration and total NPP (Wang *et al*., 2003). Comparing our calculated GPP to simulated and observed values at our sites enabled us to test for the robustness of this approach.

To support our interpretation of the rainfall and GPP time-series analysis, we employed an additional approach whereby we estimated the actual evapotranspiration (AET) as a function of a site's aridity index (i.e. PET/P *sensu* Budyko, 1974). PET was calculated based on temperature and day-length (Hamon, 1963), and setting the fraction of day that is day-time to 0.5. AET was calculated as the difference between annual precipitation and streamflow (Jones *et al*., 2012). Streamflow data from Rio Mameyes (USGS ID 50065500) and Rio Cerrillos (USGS 50114000) were used for LUQ and SUS, respectively. Budyko (1974) and many others thereafter (e.g. Potter *et al*., 2005; Gerrits *et al*., 2009) have shown that catchments where monthly potential evaporation (including transpiration) and precipitation rates are in-phase plot closer to or above the Budyko curve than sites that are out of phase. The Budyko curve approach, therefore, was applied to test whether the LUQ and SUS sites are indeed 'in phase' or 'out of phase'.

Environmental waters and plant water uptake

Rainfall amounts in LUQ were measured at the nearby (482 m) Bisley watershed meteorological station, situated ~275 m above the soil- and vegetation sampling sites. Rainfall and stream water samples were collected in the Mameyes watershed as part of the long-term stable isotope monitoring programme by the United States Geological Survey (USGS) following the collection methodology outlined by Scholl *et al*. (2009). Groundwater stable isotope data from a network of wells (depths 70–100 cm) (see McDowell *et al*., 1992) sampled at stream bank and upslope positions in the nearby Bisley watershed in LUQ were also used. At the SUS site, rainfall data from the closest weather station (30 km) in the municipality of Ponce were used, while groundwater isotope estimates for this site were derived from the simulations of Jasechko *et al*. (2014).

Local meteoric water lines (LMWLs) for the LUQ site were plotted to compare with the stable isotopic distributions in rainfall, xylem water, soil water, stream water and groundwater in dual isotope space. At SUS, the LMWL was derived from near-monthly rainfall isotopic values in the Guanica Dry Forest (Govender *et al*., 2013), 14 km from the sampling site.

Samples of precipitation, from January 2008 to March 2013, were analysed for δD and $\delta^{18}O$ in the USGS Reston Stable Isotope Laboratory in Virginia, USA using either isotope ratio mass spectrometry (IRMS) or cavity ring-down spectroscopy (CRDS) (data in Scholl *et al*., 2014). Samples of xylem water, bulk soil water, and stream water were analysed for δD and $\delta^{18}O$ at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) at the University of Utah, USA, using cryogenic vacuum distillation and isotope ratio infrared spectroscopy (IRIS) on a Picarro CRDS. No spectral interference was observed when using the IRIS technique. To address any concerns about potential errors when using the IRIS technique instead of the traditional IRMS technique (West *et al*., 2010), we randomly selected samples for comparison of the two methods. The randomly selected samples were compared to a CO_2 equilibration method on the IRMS. Results of the comparison showed that the values generated from both techniques were not significantly different (values ranged from 0.2 to 0.6‰ $\delta^{18}O$) for both plant xylem water and bulk soil water samples, with an inter-technique correspondence close to unity.

We use conventional notation for isotope composition (Coplen, 2011) where $\delta^{18}O$ or $\delta^{2}H = [(R_{sample}/R_{SMOW}) - 1]$, with R as the ratio of $^{18}O/^{16}O$ or $^{2}H/^{1}H$ in the sample or in Standard Mean Ocean Water: SMOW. Laboratory precision (1SD) for the Picarro CRDS at SIRFER was no greater than 1.1 and 0.2‰ for δD and $\delta^{18}O$, respectively, and no greater than 1 and 0.1‰ at the USGS Reston Stable Isotope Laboratory.

To understand the depth of soil water-uptake patterns, xylem water in mahogany trees and bulk soil water were collected during a relatively 'wet' (9–13 July 2012) and a relatively 'dry' (11–15 February 2013) sampling period. Samples of stream water were also taken at this time to examine to what extent it differed isotopically from bulk soil water and xylem water. Xylem water samples were taken from the part of twigs with mature bark that were closest to the main branch (following Dawson, 1993) to minimize the effect of evaporative enrichment by water loss through unsuberized stems. Xylem water was analysed for $\delta^{18}O$ and $\delta^{2}H$. We calculated the classic

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deuterium-excess parameter values (Dansgaard, 1964) and report these for soils, for comparison to previous evaporation studies (e.g. Simonin *et al.*, 2013):

$$d\text{-excess} = \delta^2H - 8\left(\delta^{18}O\right) \quad (1)$$

In addition, to test for ecohydrological separation, we calculated the line-conditioned excess (lc-excess*) of soil water, xylem water, and groundwater (Landwehr and Coplen, 2006):

$$lc-excess^* = \left[\delta^2H - a\,\delta^{18}O - b\right] / S \quad (2)$$

where a and b are the slope and y-intercept, respectively, of the LMWL, and S is one standard deviation measurement uncertainty for both $\delta^{18}O$ and δ^2H. Equation 2 was used to quantify the degree of 'offset' of environmental waters from rainfall. That is, a negative offset that is greater than the standard deviation of the LMWL suggests that water has undergone some evaporative isotopic enrichment.

Soil cores were extracted at a distance of approximately twice the average diameter at breast height (DBH) from each tree [0.62 ± 0.32 m at LUQ *versus* 0.41 ± 0.29 m at SUS (mean ± 1SD)]. Cores were taken down to depths of 30 and 60 cm during the July 2012 (wet conditions) and February 2013 (dry conditions) sampling campaigns, respectively. Cores were subdivided into 10-cm depth intervals for subsequent water stable isotope analysis. *d*-excess values at each depth in the soil profile were then calculated for both sampling periods. For LUQ we calculated the so-called characteristic length L_C for Stage 1 evaporation (i.e. the 'constant rate period', Or *et al.*, 2013) to see how the patterns of *d*-excess values with soil depth compared with L_C. L_C was calculated over a range of apparent soil water evaporation rates e_0 of sites considered representative of conditions prevailing in the forest at LUQ (0.14–0.19 mm d^{-1}, Jordan and Heuveldop, 1981; Roche, 1982):

$$L_C = \frac{L_G}{1 + \frac{e_0}{K_{eff}}} \quad (3)$$

where K_{eff} (mm d^{-1}) is effective soil hydraulic conductivity and L_G (mm d^{-1}) is the gravity characteristic length (following Or *et al.*, 2013):

$$L_G = \frac{1}{\alpha(n-1)}\left(\frac{2n-1}{n}\right)^{\left(\frac{2n-1}{n}\right)}\left(\frac{n-1}{n}\right)^{\frac{1-n}{n}} \quad (4)$$

where α and n are the van Genuchten model parameters for the silty clay soils in LUQ. We state a caveat that the e_0 estimates used here are based on micro-lysimeters (Jordan and Heuveldop, 1981) and evaporation pans placed beneath the rain forest canopy (Roche, 1982), and therefore driven by atmospheric parameters, notably temperature, wind speed, relative humidity, and solar radiation. In contrast, the derivation of Equation 3 is driven not by atmospheric parameters but by porous media properties (Lehmann *et al.*, 2008). Calculating L_C, therefore, was done to serve as a learning tool and to derive potential insights on soil water evaporation as indicated by the obtained patterns of *d*-excess values with soil depth.

Finally, to determine the sources of water uptake by the mahogany trees at different landscape positions, we employed an SLM Bayesian model approach. Ridge-top trees in LUQ were situated 95 m away from the stream valley on a slope of 12%, while ridge-top trees in SUS were situated 243 m away from the stream valley on a slope of 16%. We used the SIAR (stable-isotope analysis in R) Bayesian mixing model statistical package (Parnell *et al.*, 2010) to explore the structure (and plausible meaning) of the data in probability space (i.e. in p-space). SIAR is widely used in food web and animal foraging studies, and was used here to determine the relative importance of various sources of water that may contribute to xylem water using Markov Chain Monte Carlo (MCMC) methods. We classified four potential sources of xylem water when running the Bayesian model: (1) soil water at 0–10 cm ('shallow soil water'); (2) soil water at ≥20 cm ('deep soil water'); (3) 'rain'; and (4) 'groundwater'. We recognize that the distinguished depths are not strictly 'shallow' and 'deep' *per se*, but the terms are used here only to designate the two soil water end-members that can be resolved by SIAR vis-à-vis our sampled soil depths. The trophic enrichment factor (TEF) and concentration dependence of the original model were set to 0. The model was run with 500 000 iterations (discarding the first 50 000) and a source water's most likely contribution (i.e. the mean of the posterior distribution of the MCMC simulation) to xylem water was obtained for all trees at a site.

RESULTS

Ecohydrologic phasing: in-phase versus out-of-phase

Figures 1a and 1b show rainfall amount (mm), estimated GPP (g m^{-2} y^{-1}), and rainfall $\delta^{18}O$ (where available) for the LUQ and SUS sites, respectively. At LUQ, our calculated total annual GPP ranged from a minimum of 5400 g m^{-2} y^{-1} in 2011 to a maximum of 5600 g m^{-2} y^{-1} in 2012. Between 2005 and 2012, the mean (±SD) calculated annual GPP was 5500 (±44) g m^{-2} y^{-1}, well within the range of simulated (Wang *et al.*, 2003) and observed (LTER-LUQ) GPP estimates at 5000 and 6000 g m^{-2} y^{-1}, respectively. Over the same period, rainfall amount and rainfall $\delta^{18}O$ (median, interquartile

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range) at LUQ were 3696 (1369) mm and −1.83 (1.8) ‰, respectively. Volume-weighted average rainfall $\delta^{18}O$ and $\delta^{2}H$ were −2.5 and −8.6‰, respectively. At SUS, our calculated total annual GPP ranged from a minimum of 3000 g m^{-2} y^{-1} in 2008 to a maximum of 3100 g m^{-2} y^{-1} in 2010. Between 2005 and 2012, calculated mean annual GPP was 3105 (±7) g m^{-2} y^{-1}. The absence of information on observed or simulated GPP estimates for the forest at SUS did not allow us to directly compare the present GPP estimates. However, at 564 (±8) g m^{-2} y^{-1}, our total NPP estimates agreed closely with the NPP of ~550 g m^{-2} y^{-1} derived for this forest type by Murphy *et al.* (1995). Over the same period, rainfall amount (median, interquartile range) at SUS was 916 (386) mm. Between 2008 and 2012, the amount-weighted average rainfall $\delta^{18}O$ and $\delta^{2}H$ were −3.5 and −17.4‰, respectively (Govender *et al.*, 2013).

Using the time-series from Figures 1a and 1b, we calculated the respective spectral density patterns (Baldocchi *et al.*, 2001) for rainfall, GPP, and rainfall isotopic composition to examine whether any periodic structure existed in the data and to identify the frequencies associated with any such periodicity. For LUQ, Figure 1c demonstrates that both GPP and rainfall isotopic composition have a periodicity (i.e. occurrence of dominant peaks) of ~12 months. There are also underlying alternative periodic components (i.e. smaller peaks) recurring about every ~7 and ~6 months for GPP and rainfall isotopic composition, respectively. Using Fisher's Kappa statistic to test the null hypothesis that the time series is drawn from a normal distribution, against the alternative hypothesis that the time series has some periodic component, we rejected the null hypothesis and confirmed the periodic components of GPP and rainfall isotopic composition in the case of LUQ (Fisher's Kappa = 16.23, $P < 0.0001$).

Rainfall amount, on the other hand, showed no apparent dominant peaks or identifiable spectral pattern with this method, (Fisher's Kappa = 5.30, $P = 0.20$). Nevertheless, LUQ rainfall has the annual pattern of the Caribbean region, with a winter dry season (Dec–Apr), an early wet season (Apr–May), a mid-summer drier period (Jun–Jul) and a late wet season (Aug–Nov) (García-Martinó *et al.*, 1996; Comarazamy and González, 2011). The variability in timing and magnitude of these 'seasons', suggests that a wet/dry bimodal pattern in rainfall may not become apparent as a signal with the same frequency (García-Martinó *et al.*, 1996). A more recent effort to identify any periodic components in rainfall at LUQ (Van Beusekom *et al.*, 2015), however, confirmed earlier research findings that rainfall in Puerto Rico generally has a periodic component with a recurrence interval between 4 and 12 months. This range corresponds reasonably well with that observed in the GPP and rainfall isotopic composition signals (Figure 1a).

At SUS, Figure 1d shows a generally similar pattern to that found for LUQ, except for a more pronounced absence of any dominant peaks in rainfall amount, and low-amplitude, smaller peaks in GPP and rainfall isotopic composition signals. Nevertheless, the results of the spectral density analyses for both sites support the observation that rainfall amount and GPP have periodic components of a few months to 1 year.

Finally, to demonstrate the temporal correspondence between rainfall amount and GPP, using the same datasets we calculated the mean monthly values of the two variables. Figures 1e and 1f show that long-term mean monthly rainfall amount correlates positively with long-term mean monthly GPP (Pearson's $r = 0.20$ for LUQ, $r = 0.22$ for SUS; $P < 0.05$ using two-tailed test). On the other hand, rainfall amount was negatively correlated with rainfall isotopic composition ($r = -0.38$ for LUQ, $r = -0.44$ for SUS; $P < 0.05$ using two-tailed test).

On an annual timescale, Figure 2 shows the calculated AET as a function of site aridity index (i.e. the ratio of potential evapotranspiration to precipitation, PET/P). Pertinently, the range of PET/P-values for SUS is much wider (1.68) than that for LUQ (0.09). On the other hand, the range of the ratio of AET to precipitation (AET/P) at SUS is narrower (0.09) than that for LUQ (0.13). Calculating the long-term average AET/P deviation from the general Budyko curve shows that LUQ has a slightly negative deviation of −0.60 (±0.28) (mean ± SD), which is statistically different (two-tailed *t*-test $P < 0.05$) from SUS's slightly positive deviation of 0.10 (±0.32) from the Budyko prediction. Taken altogether, the calculated

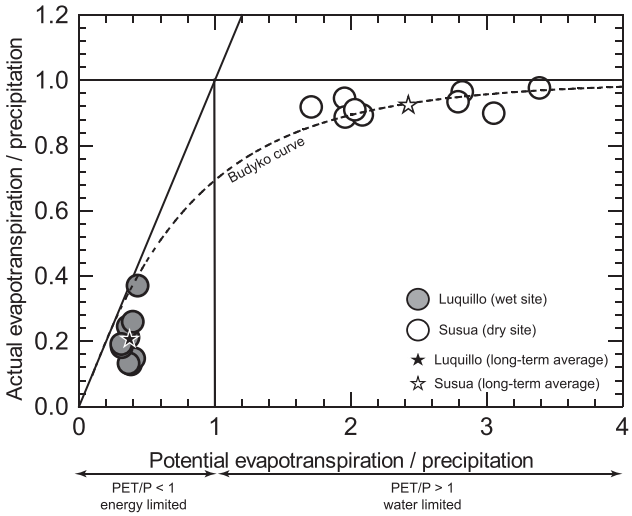

Figure 2. Inter-annual ecohydrologic variability and the Budyko curve. AET/P *versus* PET/P calculated between 2005 and 2013. AET = actual evapotranspiration; PET = potential evapotranspiration; P = precipitation. PET was calculated after Hamon (1963). Data sources: Luquillo Critical Zone Observatory website for LUQ; Weather Underground for SUS; USGS stations 50065500 and 50114000, respectively

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AET/P at both sites are in excellent agreement with the Budyko curve's prediction ($r^2 = 0.98$, $P < 0.0001$), suggesting that rain inputs and plant physiological behaviour are 'in phase'.

Ecohydrological separation: line-conditioned excess

Stable isotope values of all water samples plotted in dual isotope space for the two study sites are shown in Figures 3a–d. The slope and intercept of the LMWL at LUQ ($\delta D = 8.59\ \delta^{18}O + 13.14$) are different from the LMWL at SUS ($\delta D = 7.79\ \delta^{18}D + 10.85$); the SUS intercept (10.85) is closer to that of the GMWL ($(\delta D = 8\delta^{18}D + 10)$) while the LUQ intercept (13.14) reflects the generally higher *d*-excess in precipitation samples there (Scholl *et al.*, 2014). At LUQ, while bulk soil and plant xylem water were isotopically distinct from stream water, groundwater, and rainfall (LMWL), this separation, as shown by the lc-excess*, was more evident during the wet period (inset Figure 3a) than during the dry period (inset Figure 3b). Table II shows the key statistical information derived from the data in Figure 3. Differences in soil and xylem water lc-excess* at LUQ were not statistically significant in either moisture period ($P > 0.05$ using two-tailed *t*-test). These soil and plant xylem water lc-excess* patterns at LUQ indicate that the variability of soil water isotopic composition – down to depths of 30 and 60 cm during the wet and the dry period, respectively – can explain a fair degree of the observed variability in xylem water composition. The difference in groundwater lc-excess* at LUQ, however, was statistically significant between the two periods with contrasting moisture status ($P < 0.0001$ using two-tailed *t*-test). Also, the lc-excess* values of soil water and xylem water were statistically different from the lc-excess* of groundwater during both periods ($P < 0.0001$ using non-parametric Steel–Dwass method).

Like at LUQ, bulk soil and plant xylem water isotopic composition was also distinct from that of groundwater and rainfall at SUS during the wet period (inset Figure 3c) and the dry period (inset Figure 3d). Differences in soil water lc-excess* at SUS were statistically significant

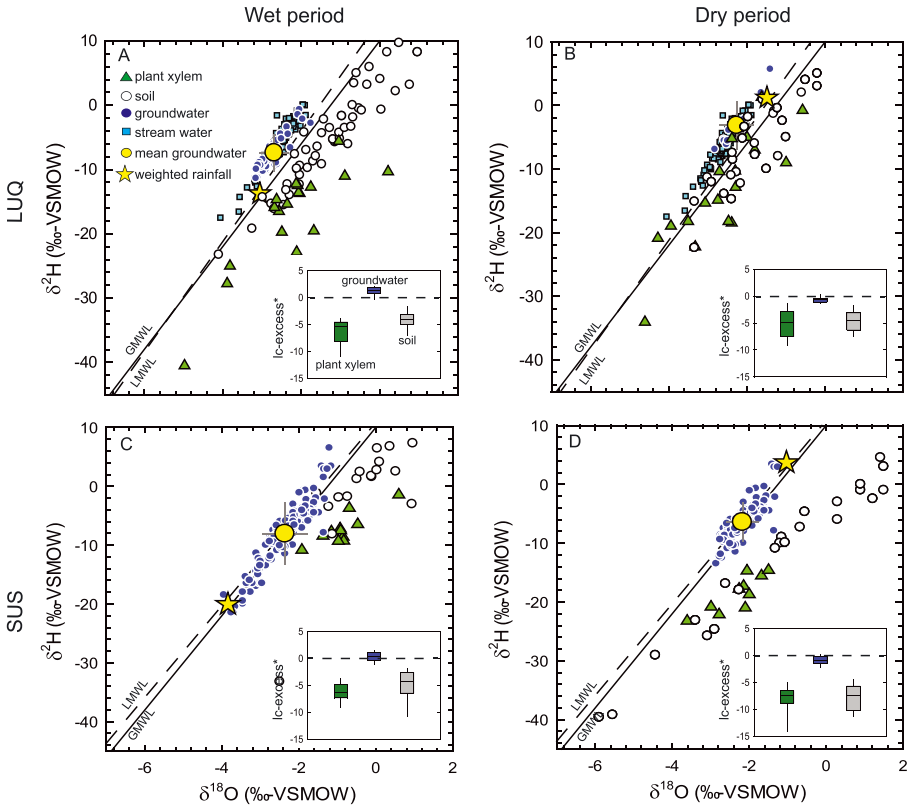

Figure 3. Dual isotope plot. (A) and (B) Stable isotopes of water from xylem (N = 18), bulk soil (N = 54 'wet period', 9–13 July 2012; N = 94 'dry period', 11–15 February 2013), stream (N = 166), and groundwater wells (N = 37 wet period; N = 12 dry period) at LUQ. (C) and (D) Stable isotopes of water from xylem (N = 12 wet period; N = 11 dry period), bulk soil (N = 33 wet period; N = 65 dry period), and groundwater (N = 31 wet period; N = 16 dry period) at SUS. LUQ LMWL: $\delta D = 8.59\delta 18O + 13.14$; SUS LMWL: $\delta D = 7.79\delta 18O + 10.85$ (Govender *et al.*, 2013). Insets show respective line-conditioned excess (lc-excess*) values (using Equation 2). All samples were taken during the rainless periods within the dates indicated. Values of groundwater and rainfall represent long-term averages of each site's respective 'wet' and 'dry' periods. The extents of the boxes show the 25th and 75th percentiles; whiskers show the extents of outliers

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Table II. Stable isotope ratios and lc-excess* of soil, xylem water, and groundwater (mean (± 1 SD))

Site	Moisture period	Bulk soil water				Plant xylem water				Groundwater			
		δ^2H	$\delta^{18}O$	lc-excess*	N	δ^2H	$\delta^{18}O$	lc-excess*	N	δ^2H	$\delta^{18}O$	lc-excess*	N
LUQ	Wet	−4 (8.3)	−1.1 (1.2)	−4.2 (1.8)	54	−17 (7.8)	−2.3 (1.2)	−6.5 (2.9)	18	−7.4 (2.8)	−2.7 (0.36)	1.1 (0.84)	37
	Dry	−6 (6.7)	−1.8 (0.89)	−4.7 (1.9)	94	−13 (8.6)	−2.6 (1.1)	−5.2 (2.8)	18	−3.1 (3.6)	−2.3 (0.42)	−0.7 (0.54)	12
SUS	Wet	5.76 (11)	0.64 (1.7)	−5.1 (3.3)	33	−8.5 (4.8)	−1 (0.68)	−6.3 (1.8)	12	−8.1 (5.2)	−2.4 (0.58)	0.26 (1.1)	150
	Dry	−14 (13)	−1.6 (2.2)	−8.1 (2.6)	65	−19 (5.5)	−2.2 (0.66)	−8.2 (2.8)	12	−6.4 (3.2)	−2.2 (0.39)	−0.9 (1.0)	120

($P < 0.00001$ using two-tailed *t*-test) between the two moisture periods. Xylem water lc-excess* values, on the other hand, were not significantly different between the two periods. Unlike at LUQ, the soil and plant xylem water lc-excess* patterns observed at SUS suggest that the isotopic variability of soil water may not explain the variability in xylem water. Like at LUQ, the lc-excess* values of soil water and xylem water at SUS were statistically different from the lc-excess* of groundwater during both the wet and the dry period ($P < 0.0001$ using non-parametric Steel–Dwass method).

Depth profiles of soil water *d*-excess values during the wet and dry periods are shown in Figure 4 while Table III lists the corresponding key statistical information. At LUQ (Figure 4a), *d*-excess values of soil water between 10 and 30 cm depth were closer to the LMWL during the dry period than during the wet period. Differences in soil water *d*-excess between the wet and dry periods were statistically significant at depths of 10 cm and 20 cm. Conversely, the difference at 30 cm depth was not statistically significant between the two moisture periods. However, the inferred magnitudes of evaporation between 10 and 30 cm depth during the wet period were not statistically different. During the dry period, a pairwise comparison of soil water *d*-excess values between 10 and 50 cm showed that only the *d*-excess values at 20 and 50 cm depth were statistically different from each other ($P < 0.0001$, non-parametric Steel–Dwass method). At 60 cm, the mean *d*-excess was highest and closest to the LMWL, while it differed statistically from values derived for all depths between 10 and 50 cm. The inset in Figure 4 a shows the modelled characteristic length (L_C) for Stage 1 evaporation (Equation 3) which suggests an L_C of ~180 cm for the low soil water evaporation rates considered applicable at LUQ (0.14–0.19 mm d^{-1}). Higher evaporation rates (~0.71 mm d^{-1}), however, would be required to explain the depth of soil water evaporation, as inferred from observed soil water *d*-excess below the LMWL value, which persisted down to 50 cm (Figure 4a).

Figure 4b shows that soil water *d*-excess patterns at SUS were different from those found for LUQ. During the wet period, soil water *d*-excess values were more positive than during the dry period. Differences in soil water *d*-excess between wet and dry periods were statistically significant at a depth of 10 cm only, but not at 20 cm and 30 cm. Similarly, the magnitude of soil water evaporation inferred from *d*-excess between 20 and 30 cm depth during the two moisture periods was not statistically different. During the dry period, comparison of differences in soil water *d*-excess between 10 and 60 cm showed that the *d*-excess values at 10 and 60 cm were statistically significant from those at 50 and 30 cm depth only ($P < 0.05$, Tukey–Kramer HSD).

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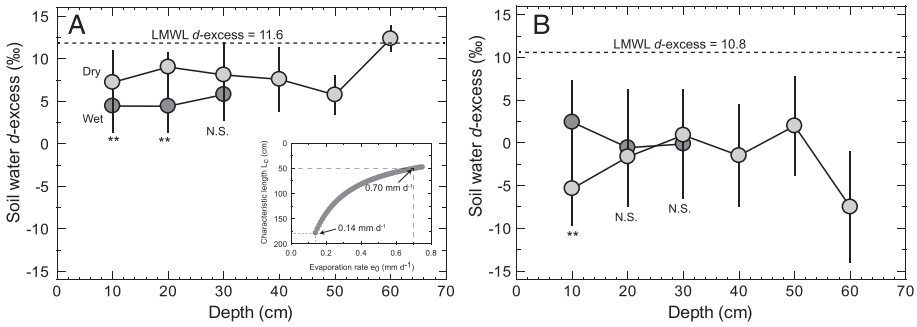

Figure 4. Soil water *d*-excess. Soil water and LMWL *d*-excess in LUQ (A) and SUS (B) during wet (dark gray circles) and dry (light gray circles) periods; error bars are 1SD; **statistically significant ($\alpha = 0.05$); N.S. Not Significant. LMWL *d*-excess was calculated using long-term annual VWA values δ^2H and $\delta^{18}O$. Inset in (A) shows the calculated evaporation rate e_0 and characteristic length L_C in LUQ using van Genuchten model parameters (after Or *et al.*, 2013). Depth sample sizes are: 10-cm N = 18, 20-cm N = 18, 30-cm N = 18 during wet period in LUQ; 10-cm N = 18, 20-cm N = 18, 30-cm N = 17, 40-cm N = 15, 50-cm N = 15, 60-cm N = 9 during dry period in LUQ; 10-cm N = 9, 20-cm N = 12, 30-cm N = 12 during wet period in SUS; 10-cm N = 12, 20-cm N = 12, 30-cm N = 12, 40-cm N = 12, 50-cm N = 12, 60-cm N = 5 during dry period in SUS

Table III. Stable isotope ratios, *d*-excess of soil water per depth, and moisture period, mean (± 1 SD)

Site	Moisture period	Depth (cm)	δ^2H	$\delta^{18}O$	*d*-excess	N
LUQ	Wet	10	−4 (9.5)	−1 (1.4)	4.4 (3.1)	18
		20	−2.6 (8.4)	−0.9 (1.3)	4.4 (3.1)	18
		30	−5.4 (6.6)	−1.4 (0.92)	5.8 (3.0)	18
	Dry	10	−1 (6.2)	−1 (0.95)	7.2 (3.7)	18
		20	−7.1 (5.0)	−2 (0.62)	9 (1.7)	18
		30	−11 (7.1)	−2.4 (0.62)	8.1 (3.8)	17
		40	−6.6 (5.8)	−1.8 (0.55)	7.6 (3.7)	15
		50	−4 (6.8)	−1.2 (0.82)	5.8 (2.2)	17
		60	−7.6 (5.4)	−2.5 (0.47)	12.3 (1.5)	9
SUS	Wet	10	8.17 (7.0)	0.73 (1.4)	2.44 (4.7)	12
		20	3.1 (16)	0.44 (2.2)	−0.6 (6.8)	12
		30	6.1 (8.6)	0.79 (1.2)	−0.1 (6.3)	9
	Dry	10	−1.8 (5.7)	0.43 (1.2)	−5.4 (4.2)	12
		20	−16 (13.2)	−1.8 (2.2)	−1.6 (5.4)	12
		30	−18 (13.8)	−2.4 (2.0)	0.92 (2.6)	12
		40	−17 (9.8)	−1.9 (1.9)	−1.5 (5.9)	12
		50	−21 (14)	−2.9 (2.4)	2 (5.8)	12
		60	−5.5 (4.1)	0.24 (1.3)	−7.5 (6.5)	5

Source water partitioning: Bayesian SLM model

Potential sources of xylem water were determined using a Bayesian mixing model approach. Figures 5 and 6 show the results for LUQ and SUS, respectively, for both wet and dry periods and all landscape positions, while Table IV lists the corresponding key statistical information. Also shown are the respective probability density plots for each end-member, superimposed on the plots of their relative contributions to xylem water. At LUQ, groundwater contribution to xylem water in ridge-top trees decreased from (mean ±1SD) 26 ± 12% during the wet period to 14 ± 12% during the dry period, while 'deep' soil water contribution increased from 27 ± 13% to 53 ± 19%, respectively. Groundwater contribution to xylem water in valley-bottom (i.e. riparian) trees increased from 25 ± 15% during the wet period to 28 ± 14% during the dry period, while rain water contribution decreased from 42 ± 18% to 21 ± 13%, respectively. Trees on slopes also showed an increase in groundwater contribution from 21 ± 13% to 29 ± 15%. At SUS, groundwater contribution to ridge-top trees increased from 23 ± 12% (wet period) to 35 ± 9% (dry period). Overall differences in source water proportions for ridge-top and valley-bottom trees at LUQ were statistically significant ($P < 0.05$) between wet and dry periods. Source water proportion differences, however, were not significant ($P > 0.05$) for all landscape positions at SUS, nor for the trees on the slope at LUQ between wet and dry periods.

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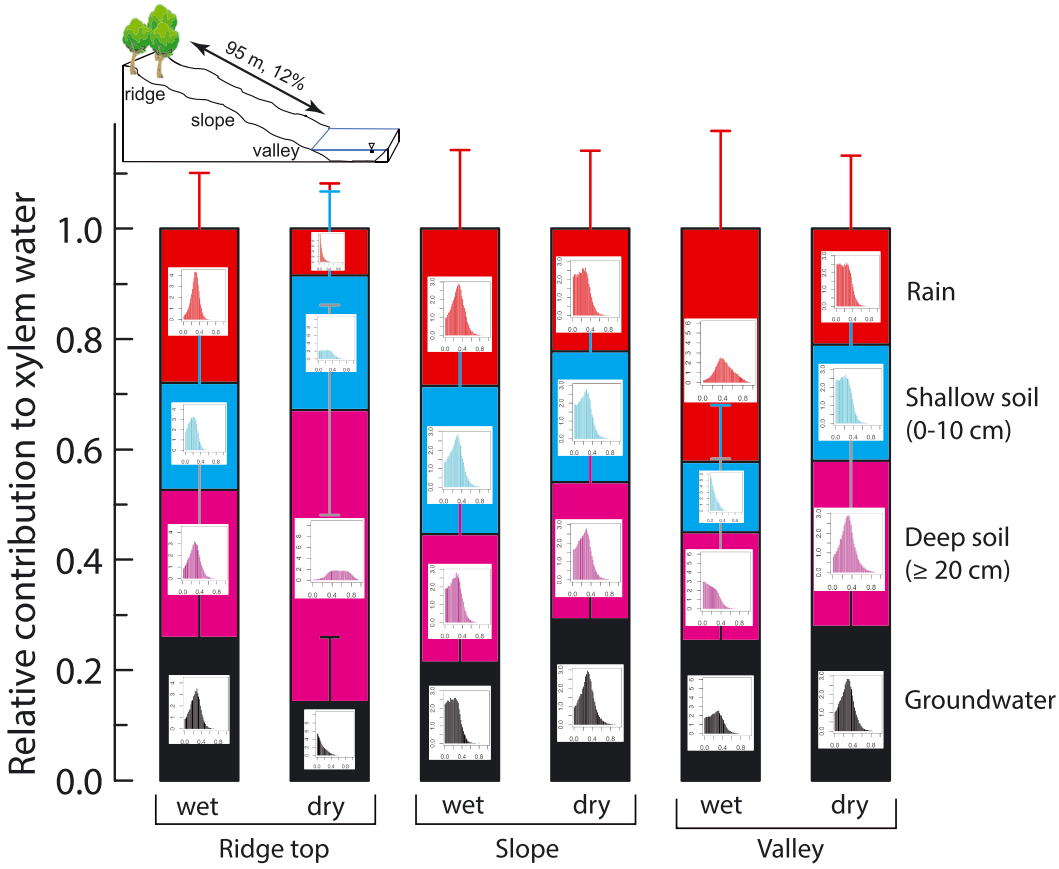

Figure 5. Source water partitioning using Bayesian mixing model (results shown for LUQ). Top-left illustration shows distance from ridgetop to stream divide and slope steepness (in percent slope). Also shown are the respective probability density plots of each putative source water superimposed on plots of relative contribution to xylem water. Error bars represent 1 SD

DISCUSSION

Stable isotope ratios of water from trees, soils, streams, wells, and rainfall were used to test for ecohydrological separation (plants using soil matrix water rather than rapidly percolating water that contributes to groundwater recharge and streamflow). Our two sites in Puerto Rico had contrasting moisture dynamics: a tropical wet forest at Luquillo (LUQ) and a tropical dry forest at Susua (SUS). Both these sites have lower seasonality (i.e. precipitation input is more 'in phase' with primary productivity) than previously studied sites for which there is evidence of ecohydrological separation between preferential flow and soil matrix water (see Evaristo *et al.*, 2015). We originally hypothesized that the plant water use patterns that would indicate ecohydrological separation as reported by Brooks *et al.* (2010) and Goldsmith *et al.* (2012) might not be equally evident at our sites where rainfall and gross primary productivity are more in-phase, because exchange between soil-matrix and preferential flow path waters may be more frequent (*sensu* Phillips, 2010). We found instead a clear separation between water forming plant transpiration and water forming groundwater recharge and/or streamflow at both our sites. We also showed how mahogany trees at our two contrasting sites may have partitioned the sources of water by demonstrating the utility of a SLM model, implemented within a Bayesian framework. In the following we will discuss the processes that may lead to evaporative isotopic enrichment of soil water; examine the state-of-the-research regarding the ecohydrological separation hypothesis; and, explore the utility of a SLM model, implemented in a Bayesian framework, in understanding source water partitioning for mahogany trees at different landscape positions and moisture periods.

Evaporative isotopic enrichment of soil water

Soil water isotope concentrations at SUS generally reflected more evaporation than those at LUQ, with greater apparent soil water evaporation during the dry period than during the wet period. We found the opposite pattern at LUQ where apparent soil water evaporation appeared to be greater during the wet period than during the dry period. Direct measurement of evaporation rates from the forest floor soil and litter (*Es*) at LUQ are not

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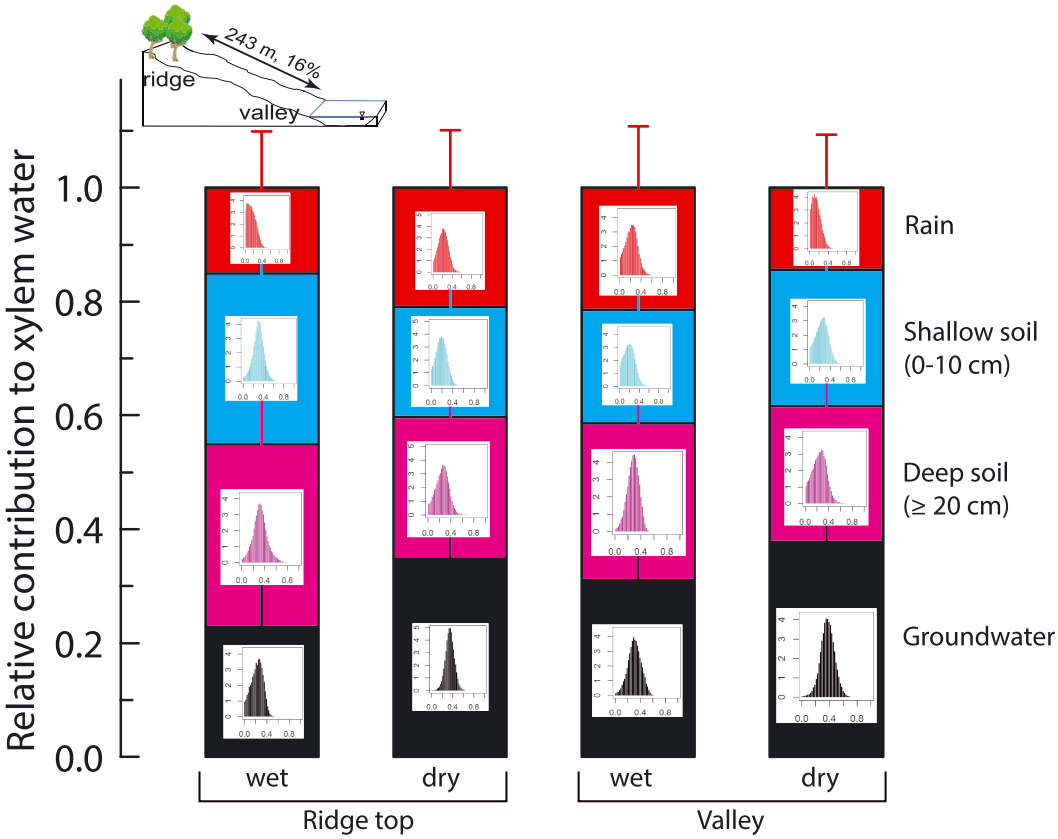

Figure 6. Source water partitioning using Bayesian mixing model (results shown for SUS). Top-left illustration shows distance from ridgetop to stream divide and slope steepness (in percent slope). Also shown are the respective probability density plots of each putative source water superimposed on plots of relative contribution to xylem water. Error bars represent 1 SD

available but annual totals observed in old-growth lowland equatorial rain forests in French Guyana (Roche, 1982) and Amazonian Venezuela (Jordan and Heuveldop, 1981) as well as in subtropical evergreen forest in South China (Liu *et al.*, 2015) ranged between 36 and 68 mm only (0.14–0.19 mm d^{-1}). Actual values of *Es* in the studied mahogany stand at LUQ may well be somewhat higher, however, given the site's location in the trade-wind belt and the occasional passage of canopy-opening hurricanes. Conversely, (very) high wet-canopy evaporation rates (*Ew*) during and shortly after rainfall have been reported for the Tabonuco forest close to where we conducted our study. Whilst estimates of *Ew* for the Bisley forest vary depending on the methodology used (Schellekens *et al.*, 2000; Holwerda *et al.*, 2006, 2012) the best estimates converge around a value of ~0.6 mm h^{-1} (Holwerda *et al.*, 2012). Such evaporation rates are well in excess of levels sustained by net radiant energy in the area (Schellekens *et al.*, 2000; Holwerda *et al.*, 2012). Instead, the observed high rates of *Ew* are thought to be maintained by a negative downward sensible heat flux from the overlying (warmer) air towards the (cooler) wetted canopy. In addition, evaporative exchange between the canopy and the atmosphere appears to be facilitated by the complex topography of the area which may lead to enhanced turbulence and thus greater aerodynamic conductance (Holwerda *et al.*, 2012). We considered whether the well documented high rates of *Ew* at LUQ would lead to throughfall with evaporated isotopic signatures. If true, such isotopically enriched throughfall would infiltrate into the soil, filling parts of the soil profile that are accessible by the roots. This might then explain the observed evaporated signal in the xylem water even with minimal soil evaporation rates. A corollary, however, is that parcels of the same evaporated throughfall input should be detectable in groundwater and stream baseflow. Our LUQ groundwater (e.g. Figure 3), stream, and long-term stream isotope data in eastern Puerto Rico (Scholl *et al.*, 2014) do not support the interpretation of a considerable contribution from throughfall with an evaporated isotopic signature. An alternative but as yet unproven explanation would be that most of the high *Ew* occurs during low-intensity rain events of long duration (cf. Schellekens *et al.*, 1999). The associated (and enriched) throughfall would be absorbed by the soil matrix and taken up by the trees. Conversely, high-intensity rain events of short duration would be less prone to enrichment by wet-canopy evaporation but

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Table IV. Bayesian mixing model results, mean (±1 SD)

		Wet period											
		Ridge				Slope				Valley			
Site		Groundwater	Deep soil	Shallow soil	Rain	Groundwater	Deep soil	Shallow soil	Rain	Groundwater	Deep soil	Shallow soil	Rain
LUQ		0.26 (0.12)	0.27 (0.13)	0.19 (0.11)	0.28 (0.10)	0.21 (0.13)	0.23 (0.14)	0.27 (0.14)	0.28 (0.14)	0.25 (0.15)	0.20 (0.13)	0.13 (0.10)	0.42 (0.18)
SUS		0.23 (0.10)	0.32 (0.12)	0.30 (0.11)	0.15 (0.10)	—	—	—	—	0.31 (0.11)	0.28 (0.09)	0.20 (0.11)	0.22 (0.11)
	Dry period												
LUQ		0.14 (0.12)	0.53 (0.19)	0.24 (0.15)	0.08 (0.08)	0.29 (0.15)	0.25 (0.14)	0.24 (0.14)	0.22 (0.14)	0.28 (0.14)	0.30 (0.15)	0.21 (0.13)	0.21 (0.13)
SUS		0.35 (0.09)	0.25 (0.11)	0.19 (0.10)	0.21 (0.10)	—	—	—	—	0.38 (0.10)	0.24 (0.12)	0.24 (0.12)	0.14 (0.09)

Remarks: Water source proportion differences in Ridge-top and Valley-bottom (i.e. riparian) trees at LUQ are statistically significant ($P < 0.05$) between wet and dry periods. However, it is not the case for all landscape positions at SUS and for the slope position at LUQ ($P > 0.05$) between wet and dry periods.

would tend to contribute to preferential flow rather than being absorbed by the matrix (cf. Schellekens *et al*., 2004). Event-based sampling for stable isotopes analysis of throughfall associated with the two types of rainstorms (cf. Te Linde *et al*., 2001) would be required to demonstrate the existence of such a mechanism.

The range of soil water evaporation rates typically found for old-growth lowland rain forests (0.14–0.19 mm d^{-1}) that we used initially to calculate the characteristic length L_C (Stage I evaporation) was also too low (i.e. calculated L_C too large) to explain the extent of evaporative enrichment inferred from the *d*-excess parameters for soil water. The inset in Figure 5a shows that higher evaporation rates (0.70–0.72 mm d^{-1}) would be required to explain the *d*-excess derived depth of soil water evaporation. Indeed, as stated earlier, it is not impossible that actual rates of soil evaporation in the studied mahogany plantation may have been somewhat higher than these initially low assumed values as the LUQ stand is likely to be better ventilated than the dense equatorial forests for which the cited rates were derived (cf. Roberts *et al*., 2005). Furthermore, the characteristic length L_C is derived from models that are 'porous media centric' rather than 'atmospherically centred'. That is, these soil-based models are informed parsimoniously by two parameters derived from the properties of the soil: the van Genuchten model parameters *n* and α. An explanation is therefore needed for the observed vertical extent of soil water with an evaporated isotopic signature—i.e. down to 50 cm. Interestingly, very little variation in soil water *d*-excess was observed between 10 cm and 50 cm (Figure 5a), suggesting that evaporative isotopic enrichment either did not systematically decrease with depth or that evaporation was restricted to the top 10 cm and transported vertically with depth. Another possibility is the mixing of rainfall having different isotopic signatures would lead to a relatively constant *d*-excess with depth. The *d*-excess values of rainfall prior to sampling, however, closely tracked the weighted rainfall *d*-excess, suggesting that mixing may not be as important a factor as evaporative enrichment. The last possibility we want to discuss is that pathways may exist for transport of water vapour from deeper pore spaces to the surface during capillary-driven Stage 1 evaporation (or similarly, during drainage) and *vice versa* during vapour-diffusion-driven Stage 2 evaporation (see Or *et al*. (2013) for discussion). One plausible way for vapour transport to persist with depth is via a subsurface architecture of soil macropores because of soil cracks, root channels, and animal burrows (as seen at LUQ by Stallard and Murphy (2012) and Larsen *et al*. (2012)) where air moves in and out of the soil system. Indeed, Silver *et al*. (1999) found that Tabonuco forest soils, where our samples were collected, remained well-aerated close to ambient O_2 concentrations of 21% down to a depth of

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35 cm, which was their maximum sampling depth. Medium-size and fine roots have also been observed at our site in LUQ down to a depth of 97 cm (USDA-NCSS, 2002). Further work on rooting depths, oxygen dynamics, and vapour transport in soils may shed some light on this topic in the future.

In addition to evaporation, root water uptake also leads to soil drying. How soil drying, as a direct result of root water uptake, affects liquid–vapour fluxes within the soil profile, however, is not well understood, nor are the effects of evaporation and root water uptake taking place in parallel with drainage (e.g. after a rainfall event) during redistribution of water within the rooting zone. Several recent studies have questioned the reliability of commonly used soil water extraction methods for isotopic analysis (Meissner *et al*., 2014; Oerter *et al*., 2014). These studies provided evidence that soil physico-chemical characteristics may play a role in isotopic fractionation, particularly with respect to $\delta^{18}O$, such that $\delta^{18}O$ in xylem water may not necessarily reflect the $\delta^{18}O$ of soil water (e.g. Geris *et al*., 2015). On the other hand, an earlier study by Ellsworth and Williams (2007) provided evidence to the contrary in that $\delta^{18}O$ in xylem water did reflect $\delta^{18}O$ in soil water. Clearly, more studies are needed to resolve the apparent issues with soil water sampling and laboratory techniques for water extraction and isotope analysis. Techniques for *in-situ*, high-frequency measurements of liquid and vapour isotopes in the unsaturated zone (Volkmann and Weiler, 2014; Sprenger *et al*., 2015; Gaj *et al*., 2016) hold great potential for exploring many of the research questions that remain unanswered with respect to ecohydrological separation (Bowen, 2015).

Ecohydrological separation research techniques: new approaches

We know, based on stable isotopes, that the water that drains through the soil profile (preferential flow water) and replenishes groundwater and streamflow is isotopically different from the residual topsoil water (soil matrix water) that roots take up for transpiration. There is now widespread, global-in-scale evidence for ecohydrological separation. The meta-analysis of Evaristo *et al*. (2015) adapted the lc-excess* method of Landwehr and Coplen (2006) while Good *et al*. (2015) used an approach that required ecohydrological separation in order to close the global water-isotope budget. These lines of evidence notwithstanding, we still lack a complete process-based understanding behind the apparent separation between topsoil water and xylem water on the one hand, and groundwater recharge and stream water on the other.

While cryogenic vacuum distillation (and now many other forms of complete water extraction) identify 'tightly bound water', clues have already been given regarding the role of mycorrhizal fungi in facilitating extraction of water held under tensions much greater than the hydrological community might expect (as reviewed by Auge (2001); Auge *et al*. (2015)) and the many papers thereafter (e.g. Allen (2007); Barzana *et al*. (2012)). Lodge (1996) reported that 98% of all trees at LUQ have roots that form symbiotic relationships with mycrorrhizal fungi to facilitate nutrient uptake from the soil. Recently, big-leaf mahogany (*S. macrophylla*) was also reported to have mycorrhizal fungi associations, with diversity that was twice greater in mature trees than in seedlings (Rodriguez-Morelos *et al*., 2014). An even more intriguing observation is that many mycorrhizal-associated plants appear to have a mechanism for extracting water below the wilting point of non-mycorrhizal-associated species (Bethlenfalvay *et al*., 1988; Franson *et al*., 1991). However, current extraction techniques prevent us from interrogating the water the plants are actually extracting for isotope analysis, both in time and space. This is a key issue for progress.

At present, we can only sample either the most mobile waters via suction lysimeters or effectively 'all the water' via cryogenic vacuum distillation (-10 to -15 MPa) or hydraulic squeezing (~41 MPa). Even more problematic is the range of spatial scales at which these current techniques are able to extract water. The range of pore sizes and subsurface architecture amenable to our extraction techniques ($10^{-5} < \text{range} < 10^{-2}$ m) is orders of magnitude greater than the scales that may be relevant to water uptake by roots ($10^{-5} < \text{diameter} < 10^{-3}$ m) and/or fungal hyphae ($10^{-6} < \text{diameter} < 10^{-5}$ m) (Smith *et al*., 2010). Moreover, the destructive nature of sampling related to these extraction techniques eliminates the opportunity to account for effects on soil properties by soil microfauna and microflora (Hallett *et al*., 2013) and *vice versa* (Kravchenko *et al*., 2013; Schwartz *et al*., 2016). Given the spatio-temporal incongruence between our soil water extraction techniques and plant (root/mycorrhizal) water uptake mechanisms, we need to develop fundamentally new extraction approaches that are able to interrogate water sources and root water uptake mechanisms at matching scales.

Ecohydrological separation and groundwater use?

Ecohydrological separation – defined as plants using water of a character different to mobile water found in soils, groundwater and streams – in no way suggests that plants do not use groundwater. There is recognition of the role of groundwater as a water source for plants when and where phreatic water is accessible (see review by Fan, 2015). Our Bayesian mixing model results show

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that groundwater contribution to dry period xylem water of valley-bottom (i.e. riparian) trees could comprise as much as $38 \pm 10\%$ and $28 \pm 14\%$ at the xeric (SUS) and mesic–hydric (LUQ) sites, respectively. Source water partitioning to riparian trees, between soil and phreatic water, is known to be influenced by moisture input fluctuations and local, or tree-level, conditions such as floodplain surface elevation and gravel layer elevation in the subsurface (Singer *et al*., 2014). These insights notwithstanding, in landscape positions (e.g. ridge-tops) at which source water partitioning results show groundwater contribution –we underline that mixing model results are dependent on the chosen end members. One plausible explanation for our mixing model-inferred groundwater use by trees on ridge tops is that deep soil water, saprolite water (Oshun *et al*., 2015), or perched groundwater within the hillslope may be isotopically the same as deeper groundwater. Synthesis of water stable isotope data may need to be cognizant of such a caveat when surveying groundwater use patterns by vegetation from regional to global scales. Nevertheless, the role of landscape position in routing and redistribution of soil water (Du *et al*., 2015) across space and time scales will need to be considered in future studies.

CONCLUSIONS

In this work we provided another line of evidence for ecohydrological separation (i.e. trees using a different water source from groundwater and streams) in two contrasting ecosystems of the less seasonal tropics in Puerto Rico. These results suggest that ecohydrological separation might be related less to temporal phase differences between hydrology (i.e. precipitation inputs) and ecology (i.e. primary productivity and water uptake by the vegetation) than with the fundamental processes that drive soil drying – e.g. soil water evaporation, root water uptake, and drainage. The interplay between the water that replenishes streamflow (preferential flow), and the water that is retained in the soil matrix for root water uptake, remains poorly understood. Future work should be focused at assessing the relative importance of these processes in both space and time; and on developing new experimental designs and methods for isotopic analysis of soil water and plant tissue. Event-based sampling for stable isotopes analysis of throughfall associated with different storm types, for example, may also prove instructive in the future. Lastly, we partitioned the sources of water by using a Bayesian mixing model. This showed that groundwater contribution to xylem water was greater for valley-bottom (i.e. riparian trees) than for ridge-top trees, and at the xeric site than at the mesic–hydric site.

DEDICATION

This work is dedicated to Frederick N. Scatena III (1954–2013), whose inspiration, guidance, support, and knowledge of the Luquillo Mountains made this work possible.

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